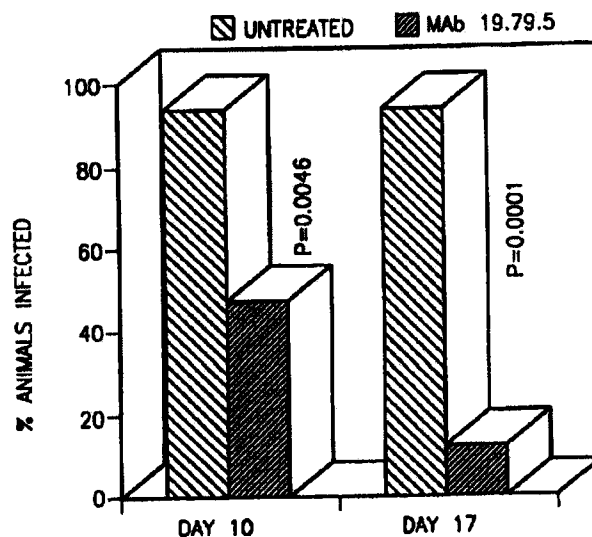


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(54) Title: HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN**(57) Abstract**

Disclosed is a process for obtaining hybridoma cell lines which produce human antibodies capable of binding to the hepatitis B virus surface antigen (HBVsAg), as well as the hybridoma cell lines, and antibodies produced by the cell lines. Also disclosed are various uses of said antibodies in the prevention and treatment of HBV infection. Peripheral blood lymphocytes obtained from human donors having a high titer of anti HBVsAg antibodies are engrafted into normal strains of mice which were lethally irradiated and radioprotected with SCID bone marrow. After immunization of such chimeric mice with HBVsAg, human cells are obtained from the mice spleens and fused *in vitro* with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

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HUMAN MONOCLONAL ANTIBODIES TO THE HEPATITIS B SURFACE ANTIGEN

FIELD OF THE INVENTION

The present invention concerns a process for obtaining hybridoma cell lines which produce human antibodies capable of binding to the hepatitis B virus surface antigen, the hybridoma cell lines, antibodies produced by the cell lines, and
5 various uses thereof.

BACKGROUND OF THE INVENTION

Hepatitis B virus (HBV) infection is a major worldwide health problem. Approximately 5% of the world population is infected by HBV and chronically
10 infected patients carry a high risk of developing cirrhosis and hepatocellular carcinoma. (Progress in Hepatitis Research: Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Hepatitis Delta virus (HDV) Ed. O. Crivelli, Sorin Biomedica, 1991).

The immune response to HBV-encoded antigens includes both a cellular
15 immune response which is active in the elimination of HBV infected cells, as well as a humoral antibody response to viral envelope antigens which contributes to the clearance of circulating virus particles. The dominant cause of viral persistence during HBV infection is the development of a weak antiviral immune response.

Recombinant HBV vaccines provide a safe and effective means for
20 active immunization against HBV, however, they do not always induce a sufficient and rapid antibody response.

Interferon- α has been used in the therapy of Hepatitis B infection showing an efficacy of only 30-40% in highly selected patients.

In addition, passive immunization with human polyclonal anti
25 Hepatitis B antisera has been shown to be effective in delaying and even preventing recurrent HBV infection (Wright, T.L. and Lau, J.Y.N. The Lancet 342:1340-1344,

(1993)). Such human polyclonal antisera are prepared from pooled plasma of immunized donors. These preparations are very expensive and available in relatively small amounts. Furthermore, pooled plasma may contain contaminated blood samples and thus treatment with such antisera increases the patient's risk to contract
5 other viral infections such as hepatitis C or HIV.

An alternative approach for the treatment of HBV infection is the use of monoclonal antibodies (MoAb).

PCT patent application PCT/NL94/00102 discloses human monoclonal antibodies directed against Hepatitis B surface antigen which are secreted by the
10 hybridoma cell lines Mab 4-7B and Mab 9H9. The monoclonal antibody secreted by the cell line Mab 4-7B recognizes a linear epitope of HBVsAg and is different from the Mab 9H9 monoclonal antibody which recognizes a conformational epitope. The antibodies are claimed for simultaneous use in the treatment of chronic Hepatitis B infections.

15 PCT patent application PCT/US92/09749 discloses human monoclonal antibodies against HBVsAg which are secreted by the hybridoma cell lines PE1-1, ZM1-1, ZM1-2, MD3-4 and LO3-3. The antibodies bind to different HBV epitopes and are used for reducing the level of circulating HBVsAg.

Japanese Patent Application JP 93066104 discloses a hybridoma of a
20 human lymphocyte cell strain TAW-925 and a human lymphocyte transformed by Epstein-Barr virus. The hybridoma produces a human monoclonal antibody against HBVsAg.

U.S. Patent Application No. 4,883,752 discloses preparation of human-derived monoclonal antibody to HBVsAg, by administration of HBVsAg
25 vaccine to humans, recovering their lymphocytes, stimulating the lymphocytes *in vitro* by a non specific stimulator, fusing said cells with a myeloma cell, and selecting for hybridomas with secrete anti HBVsAg antibodies.

Ichimori *et al.*, *Biochem. and Biophysic. Research Communications* 129(1):26-33, 1985 discloses a hybridoma secreting human anti HBVsAg
30 monoclonal antibodies which recognize the a-determinant of HBVsAg. Later, Ichimori, *et al.*, *supra* 142(3):805-812, 1987 disclosed another hybridoma which stably secretes human monoclonal antibody against HbsAg.

The abovementioned antibodies were all developed by *in vitro* immortalization of antibody-producing cells from individuals positive for anti-HBV
35 antibodies.

A new approach enabling adaptive transfer of human peripheral blood mononuclear cells (PBMC) into lethally irradiated normal strains of mice radioprotected with severe combined immune deficiency (SCID) bone marrow was recently described (Lubin I., *et al.*, *Blood*, 83:2368, 1994). Secondary humoral
5 responses to various recall antigens as well as a primary humoral response to other antigens were shown to be generated effectively in such human/mouse chimeras (Marcus H., *et al.*, *Blood*, 86:398-406, 1995).

SUMMARY OF THE INVENTION

10 In accordance with the present invention, it was found that hybridoma cell lines secreting human antibodies capable of binding to the Hepatitis B surface antigen (HBVsAg) may be obtained using the above mentioned human/mouse chimeras. In accordance with the present invention, human peripheral blood lymphocytes (PBL) from human donors positive for anti HBVsAg antibodies are
15 engrafted into normal strains of mice which were lethally irradiated and radioprotected with SCID bone marrow. After immunization of such chimeric mice with HBVsAg, human cells are obtained from the mice spleens and fused *in vitro* with heteromyeloma cells to generate hybridomas secreting human antibodies having a high affinity and specificity to HBVsAg.

20 The present invention thus provides a process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:

- (a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen (HBVsAg) such that xenogeneic
25 antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed, said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human
30 M3;
- (b) removing and immortalizing said antibody-producing cells;
- (c) selecting and cloning the immortalized antibody producing cells producing the antibodies capable of binding to HBVsAg and;
- (d) isolating the antibodies produced by the selected, cloned immortalized
35 antibody producing cells.

In accordance with the invention, spleens of the immunized chimeric rodent M4 are removed between 12 and 20 days after human PBL transplantation, preferably at day 14 after transplantation thereof. Cell suspensions are prepared from the spleens and the antibody producing cells obtained from the immunized
5 chimeric rodent M4 are fused preferably with a human-mouse fusion partner such as a heteromyeloma by techniques well known in the art (e.g. Kohler & Milstein, *Nature*, 256:495-497, 1975). In order to isolate the antibodies produced by the selected hybridoma cell lines in accordance with the invention, the hybridoma cell lines are either cultured *in vitro* in a suitable medium wherein the desired
10 monoclonal antibody is recovered from the supernatant or, alternatively, the hybridoma cell lines may be injected intraperitoneally into mice and the antibodies harvested from the malignant ascitis or serum of these mice. The supernatant of the hybridoma cell lines are first screened for production of human IgG antibodies by any of the methods known in the art such as enzyme linked immunosorbent assay
15 (ELISA) or radioimmuno assay (RIA). Hybridomas testing positive for human IgG are then further screened for production of anti HBVsAg antibodies by their capability to bind to HBVsAg.

The M1 rodent in accordance with the invention is preferably a rodent conventionally used as a laboratory animal, most preferably a rat or a mouse.
20 The mouse M2 may have any hematopoietic deficiency including genetic hematopoietic deficiencies as well as induced hematopoietic deficiencies. Non limiting examples of hematopoietic deficiencies include SCID, Bg, Nu, Xid or mice having any combination of the abovementioned hematopoietic deficiencies. In addition, the hematopoietic deficiency may also be a result of gene deletion or
25 transgenic mice may be used.

The hematopoietic cells derived from the donor mouse M2 are preferably bone marrow cells either untreated or depleted of T cells. Other suitable sources of hematopoietic cells which may also be used include, for example, spleen cells, fetal liver cells or peripheral blood cells.

30 The xenogeneic hematopoietic cells derived from the human M3 are preferably PBL cells but may also be derived from any suitable source of human hematopoietic cells such as bone marrow cells, cord blood cells, thymus spleen or lymphnode cells, etc.

By a most preferred embodiment, the rodent M1 is a mouse or rat, the
35 mouse M2 is a SCID mouse and the xenogeneic hematopoietic cells derived from the human M3 are PBLs from a human M3 which has already been exposed to the

HBVsAg either spontaneously as a result of a prior infection or induced following vaccination. Such humans will have a relatively high titer of anti HBVsAg antibodies as compared to individuals which have never been infected with HBV and, therefore, when PBLs from such donors are used as M3 donor cells in accordance with the present invention, the immunization of the M4 chimeric mouse with HBVsAg will elicit a secondary immune response of the transplanted human PBLs in the M4 chimeric mouse. A most preferred human donor M3 is such which tested negative for the HB virus but shows a high titer of antibodies against HBVsAg. Such PBLs from the human M3 donor may be obtained either by whole blood donation or by leukophoresis.

The HBVsAg used for immunizing the chimeric rodent M4 in accordance with the invention is preferably a Hepatitis B virus vaccine containing the purified major surface antigen of the virus prepared by recombinant DNA technology (Engerix™-B, SIB Biological (Rixensart, Belgium)).

The present invention is also directed to hybridoma cell lines producing human monoclonal antibodies capable of binding to HBVsAg, as well as to human monoclonal antibodies capable of binding to HBVsAg and fragments thereof substantially maintaining the antigen binding characteristics of the whole antibody. Such fragments may be, for example, Fab or F(ab)₂ fragments obtained by digestion of the whole antibody with various enzymes as known and described extensively in the art. The antigenic characteristics of an antibody are determined by testing the binding of an antibody to a certain antigenic determinant using standard assays such as RIA, ELISA or FACS analysis.

Typically, the human monoclonal antibodies obtained by the method of the present invention have a relatively high affinity to HBVsAg being in the range of about 10⁻⁹M to about 10⁻¹⁰M as determined in a competitive ELISA assay.

In accordance with a specific embodiment of the present invention there are provided hybridoma cell lines designated herein as "18.5.1013" and "19.79.5" which were deposited on May 22, 1996, in the European Collection of Cell Cultures (ECACC, CAMR, Salisbury, Wiltshire, SP40JG, U.K.) under Accession Nos. 96052170 and 96052168, respectively. Anti HBVsAg human monoclonal antibodies secreted by the above hybridoma cell lines and designated herein as "Ab18.5.1013" and "Ab19.79.5", respectively, are also provided as well as fragments thereof retaining the antigen binding characteristics of the antibodies, and antibodies capable of binding to the antigenic epitope bound by "Ab18.5.1013" and

"Ab19.79.5".

The antigen bound by the antibodies defined above also constitutes an aspect of the invention.

Further aspects of the present invention are various diagnostic,
5 prophylactic and therapeutic uses of the human anti HBVsAg monoclonal antibodies and the Ag bound by them. In accordance with this aspect of the invention, pharmaceutical compositions comprising the human anti HBVsAg monoclonal antibodies may be used for the treatment of chronic Hepatitis B patients by administering to such a patient a therapeutically effective amount of the
10 monoclonal antibody or portion thereof capable of binding to the HBVsAg being an amount effective in alleviating the symptoms of the HBV infection or reducing the number of circulating viral particles in an individual.

Such pharmaceutical compositions may comprise one or more antibodies of the invention. In addition to the antibodies of the invention the pharmaceutical
15 compositions may optionally also comprise a carrier selected from any of the carriers known in the art. One example of such a carrier is a liposome. The pharmaceutical compositions of the invention may also comprise various diluents and adjuvants known per se.

The compositions of the invention may be administered by a variety of
20 administration modes including parenterally, orally etc.

Compositions comprising the antibodies of the invention, as described above, may be administered in combination with other anti viral agents. Such agents may include, as a non limiting example: Interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogs, and inhibitors of DNA
25 polymerase. In the case of such a combination therapy the antibodies may be given simultaneously with the anti viral agent or sequentially either before or after treatment with the anti viral agent.

The pharmaceutical compositions of the invention may also be used, for example, for immunization of new born babies against HBV infections or for immunization of
30 liver transplantation patients to eliminate possible recurrent HBV infections in such patients.

By a further embodiment, the antibodies of the invention may also be used in a method for the diagnosis of HBV infections in an individual by obtaining a body fluid sample from the tested individual which may be a blood sample, a lymph
35 sample or any other body fluid sample and contacting the body fluid sample with a human anti HBVsAG antibody of the invention under conditions enabling the

formation of antibody-antigen complexes. The level of such complexes is then determined by methods known in the art, a level significantly higher than that formed in a control sample indicating an HV infection in the tested individual. In the same manner, the specific antigen bound by the antibodies of the invention may also be used for diagnosis. In the same manner, the specific antigen of the invention may also be used for diagnosis of HBV infection in an individual by contacting a body fluid sample with the Ag and determining the presence of Ag-Ab complexes in the sample as described above. In addition, the Ag of the invention may be used for immunizing an individual to elicit a humoral response against HBV.

- 10 The present invention further provides a kit for use in the therapy of HBV infections or diagnosis of such infections comprising the antibodies of the invention, the antigen bound by the antibodies of the invention and any further reagents necessary for detecting such antibodies or antigens in a tested sample.

15 **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a graphic representation showing the amount of total human Ig (mg/ml) and the amount of specific anti HBs antibodies (mU/ml) in the sera of irradiated mice which were radioprotected with SCID bone marrow (chimeric mice). PBL+Engerix: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Engerix B in an aluminum hydroxide adjuvant (alum).

- 20 PBL+Alum: the chimeric mice were further transplanted with human PBL from donors positive for anti HBs antibodies, and vaccinated with Alum alone (no Engerix B).

- 25 SCID-BM+Engerix: the chimeric mice were vaccinated with Engerix B (no transplantation of human PBL).

SCID-BM+Alum: the chimeric mice were vaccinated with Alum (no human PBL and no Engerix B).

- 30 The black line represents the initial level of anti HBs antibodies in the serum of the human PBL donor.

Fig. 2 is a graphic representation showing the specific activity, i.e. the levels of anti HBVs antibodies per mg of human Ig in the sera of human donors (A-D, black columns) and the specific activity in the sera of chimeric mice transplanted respectively with human PBL of said donors (A-D, striped columns).

- 35 Fig. 3 is a graphic representation showing time response curve of anti HBs antibodies specific activity (mU/mg) in sera of chimeric mice (dotted line). The

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black columns represent the level of total human Ig (mg/ml), and the striped columns represent the level of specific anti HBs antibodies (mU/ml).

Fig. 4 is a graphic representation showing competitive inhibition of binding of anti HBs antibodies to HBs particles. The extent of binding was measured by ELISA using a horseradish peroxidase labeled anti human IgG secondary antibody. The anti HBs antibodies were diluted as indicated in the graph in medium (empty squares) or in 0.5 µg/ml HBs particles (black squares).

Fig. 5 is a photograph showing Hepatitis B infected liver sections stained with anti HBVs antibodies. All sections were stained with a "secondary" antibody, i.e. goat anti human Ig conjugated to biotin.

A - negative control. No first antibody.
B - positive control. First antibody - mouse anti HB antibody and a secondary anti-mouse Ig.

C - staining with anti HBs antibody No. 19.79.5.
D - staining with anti HBs antibody No. 18.5.1013.

Reference will now be made to the following Examples which are provided by way of illustration and are not intended to be limiting to the present invention.

Fig. 6 is a schematic representation of the binding of Ab 19.79.5 to a set of 15 well characterized HBsAg types. The y axis represents optical density units. The x axis represents different HBsAg types.

Fig. 7 is a graphic representation of the percentage of HBV infected animals at days 11 and 18 in the untreated group and Ab 18.5.1013 treated group (in the inhibition model).

Fig. 8 is a graphic representation of the percentage of HBV infected animals at days 10 and 17 in the untreated group and Ab 19.79.5 treated group (in the combined prophylaxis/inhibition model).

Fig. 9 is a graphic representation of the percentage of HBV infected animals at days 11 and 19 in the untreated group and Ab 19.79.5 treated group (in the combined inhibition/treatment model).

Fig. 10 Nucleic acid sequence and corresponding amino acid sequence of the light chain of the variable domain of Ab 19.79.5.

Fig. 11 Nucleic acid sequence and corresponding amino acid sequence of the heavy chain of the variable domain of Ab 19.79.5.

EXAMPLES**MATERIALS AND METHODS****Mice:**

- 5 Animals used were 6-10 weeks old. BALB/c mice were obtained from Harlan (Weizmann Institute Animal Breeding Center (Rehovot, Israel)), SCID/NOD mice from the Weizmann Institute Animal Breeding Center (Rehovot, Israel). All mice were fed sterile food and acid water containing ciprofloxacin (20 µg/ml) (Bayer, Leverkusen, Germany). Whenever necessary, mice were injected daily with
- 10 1 mg Fortum i.p. for five days post BMT (Glaxo Operations UK, Greenford, England).

Conditioning Regimens:

- BALB/c mice were exposed to total body irradiation (TBI), from a
- 15 gamma beam 150-A 60Co source (produced by the Atomic Energy of Canada, Kanata, Ontario) with F.S.D of 75 cm and a dose rate of 0.7 Gy/min, with 4 Gy followed 3 days later by 10-11 Gy (split dose).

Preparation and Transplantation of Bone Marrow Cells:

- 20 The femoral and tibial bones were removed from mice and homogenized in a sterilized 50 ml Omni-Mixer stainless steel chamber (Omni-Mixer Homogenizer, Model No. 17106, OMNI International, Waterbury, CT. USA).

- Recipient mice were injected i.v. with $4-6 \times 10^6$ of SCID/NOD bone marrow cells
- 25 (in 0.2 ml PBS) immediately after irradiation.

Transplantation of Peripheral Blood Lymphocytes:

- Peripheral blood lymphocytes (PBL) were obtained after informed consent by leukaphoresis from donors positive for HBs antibodies and negative for
- 30 HBV. PBLs were washed twice, counted and resuspended in PBS to the desired cell concentration.

100×10^6 human PBL were injected intraperitoneally (i.p.) into recipient mice, conditioned as described above. Control mice did not receive human PBL.

Immunization of the Chimeric Animals:

Mice were immunized once with hepatitis B vaccine (Engerix™-B; SB Biologicals Rixensart, Belgium) administered i.p. together with the PBL.

5 Cell and Plasma Collection from Human Mouse Chimera:

Animals were bled from the retro-orbital vein using heparin-coated glass capillaries. Plasma was kept for human-Ig determination. Spleens were removed after the animals were sacrificed by cervical dislocation, cut into pieces and pressed through stainless steel sieves to make a cell suspension in PBS.

10

Cell Fusion:

Cells were mixed with the human-mouse heteromyeloma HMMA2.11TG/0 (Posner *et al. Hybridoma*, 6:611-625, 1987) at 3:1 ratio. Fusion was performed with 50% (w/v) PEG 1500 (Boehringer Mannheim GmbH) in a standard procedure. Fused cells were seeded at a concentration of 3000 cells/well in 96-well U-bottom microtiter plates (Nunc, Denmark) in complete medium containing HAT-supplement (1x) (Biological Industries, Beit Haemek, Israel). Cells were fed with fresh HAT-medium a week later. Two weeks after fusion supernatants were harvested for ELISA and medium was replaced with fresh HT-medium.

20

Hybridoma cultures secreting specific anti-HBs Ig were cloned at 0.5 cell/well in 96-well U-bottom microtiter plates.

Determination of Human Immunoglobulin:

Sera were tested for antigen specific and total human Ig. Total human Ig was quantified by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) as the capture agent and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent. Human serum of known immunoglobulin concentration was used as the standard (Sigma, Rehovot, Israel). Microplates (Nunc, Roskilde, Denmark) pre-coated with the capture reagent (2.5 ug/ml, 50 ul/well) and blocked with 1% BSA were incubated overnight at 4C with dilutions of plasma from 1:20000 to 1:640000, or the standard from 0.2 to 0.06 ug/ml, then washed 5 times with PBS-Tween solution. The detection reagent was added and the plates were incubated for 1h at 37C, then washed again 3 times. Fresh substrate solution (TMB, Sigma) was added and, after peroxidase-catalyzed color development, the reaction was stopped by addition of 10% sulfuric acid. Absorbance at 450 nm was quantified on an ELISA reader (Dynatech, Port Guernsey, Channel Islands, UK).

Concentration of antigen-specific human antibodies in mice sera was determined by HBsAb EIA kit (ZER, Jerusalem, Israel).

Human antibodies in hybridoma supernatants were determined by overnight incubation of supernatants on goat anti-human IgG+A+M (Zymed) coated plates, with goat anti-human IgG-peroxidase conjugated as the secondary reagent.

Antigen-specific antibodies in hybridoma supernatants were determined as above using Hbs antigen coated plates.

Determination of Human IgG Subclasses:

Human IgG subclasses were determined by sandwich ELISA using goat F(ab)2-purified anti-human IgG+IgM+IgA (Zymed Laboratories, San Francisco, CA) coated plates and Hbs antigen coated plates. Mouse anti-human IgG subclasses (Sigma) were used as second antibody and peroxidase-conjugated purified goat anti-human (Zymed Laboratories) as the detection reagent.

Statistic Analysis:

Statistical analysis was performed using the Stat View II program (Abacus Concepts, Inc., Berkeley, CA) on a Mackintosh Quadra 605 or Microsoft Excel 5.0 (Microsoft) on a 486 DX2 PC compatible. Student t-test, Anova correlation and regression analysis were utilized to calculate probability (p) and correlation coefficient (r) values. Results are presented as mean \pm standard error.

Affinity Constant Measurements:

Determination of affinity constants (K_D) of the different anti-HBs antibodies to ad antigen (Chemicon Cat. No. AG 850) in solution were performed according to Friguet *et al.* (*Journal of Immunological Methods*, 77:305-319, 1985). The antigen at various concentrations ($3.5 \times 10^{-10} \text{M}$ to $1.4 \times 10^{-9} \text{M}$) was first incubated in solution with a constant amount of antibody ($3.4 \times 10^{-11} \text{M}$), in 0.1 M sodium phosphate buffer containing 2 mM EDTA and 10 mg/ml BSA, pH 7.8 (medium buffer). After o.n. incubation at 20 °C the concentration of free antibody was determined by an indirect ELISA. A volume of 300 µl of each mixture were transferred and incubated for 2h at 20 °C into the wells of a microtitration plate (Nunc) previously coated with Ad (50 µl/well at 1 µg/ml in 0.1 M NaHCO_3 buffer, pH 9.6 for 2 h at 37°C). After washing with PBS containing 0.04% Tween 20, the bound antibodies were detected by adding HRP-F(ab')₂ Goat anti human IgG (Zymed) diluted 1:3000 with medium buffer, 50 µl/well 2 h at 20°C. The plate was developed with TMB chromogen (Sigma T-3405 tablets) 50 µl/well, the reaction stopped with 10% H_2SO_4 50 µl/well and the plate read in an ELISA reader at 450 nm. The conditions were chosen so that the resulting f values (see Friguet *et al.*) were around 0.1. The antibody concentration used was deduced from an ELISA calibration done on the same plate. The affinity constant K_D was calculated from the relevant Scatchard plot.

Inhibition Assays:

The inhibition assay was performed in microtiter plates coated with HBs particles (2 µg/ml in PBS). The plate was blocked with 3% BSA in PBS. Hybridoma supernatants containing anti HBs antibodies were serially diluted. 50 µl of each dilution were added to the coated microtiter wells. Subsequently, 50 µl of HBs particles (ad/ay, 0.5 µl/ml in PBS) or PBS alone were added to each well. The plates were incubated overnight at room temperature in a humid chamber and washed 5 times with PBS-Tween. Next, 50 µl of goat anti human IgG conjugated to HRP (diluted 1:5000 in PBS) were added to each well. After a 4 hour incubation at room temperature in a humid chamber the plates were washed 5 times with PBS-Tween, and TMB was added to each well. Results were read using an ELISA reader, in a wavelength of 450 nm.

Immunohistostaining:

HBV positive liver fragment was fixed in 4% neutral buffered formaldehyde for 24 h and then embedded in paraffin using routine procedures.

- 5 Sections of 4 μ m thickness were cut from paraffin blocks and mounted on polylysine-coated slides. After deparaffinization and peroxidase quenching staining was performed using our monoclonal Human anti-HBs Protein A-purified antibodies followed by biotinylated Goat anti-Human IgG (H + L) (Zymed, San Francisco, CA) using Histostain-SPTM kit (Zymed) according to the manufacture's
10 recommendation. Control slides without using the 1st Human anti-HBs antibody were stained in parallel.

Sequence analysis:

- Total RNA was isolated from 10×10^6 hybridoma cells with RNAsol B reagent (TEL-TEX, Inc. Friendswood, Texas). cDNA was prepared from 10 μ g of
15 total RNA with reverse transcriptase and oligo dT (Promega, Madison, WI) according to standard procedures. PCR was performed on 1/50 of the RT reaction mixture with V_H , V_λ or V_κ 5' leader primers and 3' primers corresponding to human constant region. The PCR fragments were cloned into pGEM-T vector
20 (Promega). The inserts were sequenced using an ABI 377 sequencing machine. Sequences were analyzed by comparison to Genbank and by alignment to Kabat sequences (Kabat et al. 1991, Sequences of proteins of immunological interest (5th Ed.) U.S. Dept. of Health and Human Services, National Institutes of Health, Bethesda, MD).

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Example 1: Production of human anti HBs antibodies in chimeric mice

- Human peripheral blood lymphocytes (PBL) from donors positive for anti HBs antibodies were implanted intraperitoneally into irradiated BALB/C mice
30 which were radioprotected by transplantation of bone marrow from SCID mice. These chimeric mice were immunized with Hepatitis B vaccine (Engerix B) to induce a secondary immune response. The production of specific anti HBs antibodies along with total human Ig secretion was measured in mice sera. Fig. 1 shows levels of total human Ig and specific anti HBs antibodies in mice sera 14 days
35 after transplantation of human PBL. Although the levels of human Ig secreted are

similar in immunized and control mice, a strong specific immune response develops in mice vaccinated with hepatitis B vaccine as compared to the control group. Comparison of the levels of specific human antibodies produced in response to the antigen in immunized mice to their levels in the donors sera, indicates a 5-10 fold increase in the mice. Moreover, the specific activity measured in mice sera, i.e. the levels of anti HBs specific antibodies per mg of human Ig secreted, is 102-104 fold higher than the specific activity observed in the donor. This increase demonstrates a very high amplification of anti HBs antibody production in response to the antigen in the chimeric mice (Fig. 2). Production of human antibodies is detectable 10 days after immunization and reaches a plateau after three weeks. The specific activity is high at day 13 after immunization and decreases thereafter (due to increase in total human Ig secretion) (Fig. 3).

Example 2: Preparation and characterization of human monoclonal antibodies against HBs

Human B cells harvested from mice spleens two weeks after immunization were fused to human- mouse heteromyeloma cells (Posner *et al. Supra*). Hybridoma cells were tested for their growth rate, total Ig secretion and specific antibody production. Control fusion experiments were performed on the donor PBL that were activated in vitro with PWM and HBVsAg. Fusion frequencies in different experiments range from $0.9 - 5 \times 10^{-5}$. Most of the growing hybridoma clones secrete human Ig of which 0.1-4 % produce specific human anti HBs antibodies. Anti-HBs secreting hybridoma cells derived from chimeric mice spleens were compared to those obtained from fusion of the donors *in vitro* activated PBL in terms of Ig type and stability as seen in Table 1 below. The majority of the hybridomas from chimeric mice were found to be IgG type and all were stable for more than 12 months. In contrast, hybridomas derived from donor PBL were mostly unstable, only one clone has been stable for more than 12 months. Two stable hybridoma clones that secrete specific human anti HBs monoclonal antibodies were characterized. As seen in Table 2 below, these antibodies were purified on a protein A column as well as on an anti human Ig - agarose column and were both found to be of IgG1 subclass. Affinity constants range from 1.3×10^{-9} M to 6×10^{-9} M as tested by competitive ELISA. Specificity was tested by competitive inhibition assay using HB surface antigen of the ad-ay (1:1) subtype (Fig. 4). Fig. 5 shows specific binding of the human MoAbs of the invention to HBV by staining human liver fragments infected with HBV.

The gene encoding the variable region of Ab 19.79.5 was isolated, fully sequenced, and its subgroups and CDRs were determined.

The antibody has a fully human Ig gene sequence as determined by alignment to Genebank sequences and Kabat protein sequences. Fig. 10 shows the nucleotide sequence of the cDNA encoding the light chain of the variable region of Ab 19.79.5 and its corresponding amino acid sequence (Sequence identification nos. 1 and 3). Fig. 11 shows the nucleotide sequence of the cDNA encoding the heavy chain of the variable region of Ab 19.79.5 and its corresponding amino acid sequence (Sequence identification nos. 2 and 4).

The sequencing data revealed that the variable region of Ab 19.79.5 consists of the subgroups V_{H3} , J_{H2} , $V_{\lambda 3}$ and $J_{\lambda 3}$.

HBV genomes are classified into six groups A to F, based on the degree of similarity in their nucleotide sequences. The genetic variability of HBV is further reflected in the occurrence of different serotypes of HBsAg. The common determinant 'a' and two pairs of mutually exclusive determinants 'd/y' and 'w/r' enable the distinction of four major subtypes of HBsAg: *adw*, *adr*, *ayw* and *ayr*. Additional determinants designated subdeterminants of w (w1 to w4) have allowed the definition of four serotypes of *ayw* (*ayw*1-4) and two serotypes of *adw*, i.e. *adw*2 and *adw*4. Additional subtype variation is added by the *q* determinant,

which is present on almost all subtypes. Its absence is marked by a 'q-' sign.

The kind of HBV serotypes recognized by Ab 19.79.5 was examined using a set of 15 different HBsAg types (Norder et al., 1992, Journal of General Virology, 73, 3141; Magnusius and Norder, 1995, Intervirology, 38, 24-34). As can be seen in Fig. 6, Ab 19.79.5 has a complex recognition pattern of the different HBsAg serotypes.

25

Example 3: Biological activity of human monoclonal antibodies against HBs

The biological activity of Ab 19.79.5 and Ab 18.5.1013 was characterized using the following HBV animal model: a mouse was treated so as to allow the stable engraftment of human liver fragments. The treatment included intensive irradiation followed by transplantation of scid (severe combined immunodeficient) mice bone marrow. Viral infection of human liver fragments was performed ex-vivo using HBV positive human serum (EP 699 235).

The animal model was used in three different modes representing various potential uses of the antibodies: inhibition of infection mode, combined prophylaxis/inhibition mode and combined inhibition/treatment.

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1. Inhibition mode - This model demonstrates the ability to use the antibody to inhibit liver infection by HBV. HBV positive human serum was preincubated with Ab 18.5.1013, followed by standard *ex-vivo* liver infection. HBV-DNA in mice sera was tested 11 and 18 days after transplantation. As seen in Fig. 7 there was a significant reduction in the percentage of infected animals in the antibody treated group as compared to the untreated group.
2. Combined prophylaxis/inhibition mode - This model represents liver transplantation. In this model mice were treated with Ab 19.79.5(10 I.U./mouse) three days before liver transplantation followed by transplantation of human liver fragments which were *ex vivo* infected with HBV in the presence of Ab 19.79.5 (100 I.U.). HBV DNA was tested in mice sera 10 and 17 days after transplantation. As can be seen in Fig. 8, there was a significant reduction in the percentage of infected animals in the treated group compared to the control group.
3. Combined inhibition/treatment mode - a) HBV positive human serum was preincubated with Ab 19.79.5 followed by standard *ex vivo* liver infection. b) Mice were treated with Ab 19.79.5 at days 0 and 7 post transplantation. HBV DNA in mice sera was tested on days 11 and 19. As can be seen in Fig. 9, the percentage of infected animals in the Ab 19.79.5 treated group was significantly reduced but rebounded about two weeks after the treatment was stopped.

Example 4: Combination therapy of human monoclonal antibodies against HBs and an anti viral agent

Using the HBV model described above, mice are treated with an anti viral drug (a nucleoside analogue, 0.5 mg/mouse/day) at days 17-20 post transplantation. A group of mice is further treated with the human monoclonal antibodies of the invention at days 19 and 20. The presence of HBV DNA in mice sera is tested on days 21 and 27.

Table 1

Stability	Anti-HBs Secretors		Source of Hybridoma Cells
	IgM	IgG	
1 stable for > 10 months 47 unstable	25 (52%)	23 (48%)	<i>In Vitro</i> Activated PBL
6 stable for > 10 months 3 unstable	3 (33%)	6 (67%)	Chimeric Mouse Splenocytes

5

Table 2

10

Kd (M)	Production mg/10 ⁵ cells/day	Type	Clone
6.1×10^{-9}	10.3	IgG1 VI	18.5.1013
1.62×10^{-9}	5.8	IgG1 VI	19.79.5

CLAIMS:

1. A process for obtaining human monoclonal antibodies (hMoAb) capable of binding to Hepatitis B virus surface antigen (HBVsAg) comprising:
 - 5 (a) immunizing a chimeric rodent M4 having xenogeneic hematopoietic cells with Hepatitis B surface antigen HBVsAg such that xenogeneic antibody-producing cells are produced in said rodent, wherein said rodent M4 is a rodent M1, the hematopoietic cells of which have been substantially destroyed,
10 said rodent M1 having transplanted therein hematopoietic cells derived from a mouse M2 having a hematopoietic deficiency, and xenogeneic hematopoietic cells derived from human M3;
 - (b) removing and immortalizing said antibody-producing cells;
 - (c) selecting and cloning the immortalized antibody producing
15 cells producing the antibodies capable of binding to HBVsAg and;
 - (d) isolating the antibodies produced by the selected, cloned immortalized antibody producing cells.
2. A process according to Claim 1, wherein the rodent M1 is a BALB/C mouse and the mouse M2 is a SCID mouse.
- 20 3. A process according to Claim 1 or 2, wherein the human M3 is human having a high level of anti HBVsAg antibody and said xenogeneic hematopoietic cells derived from human M3 are peripheral blood lymphocytes (PBL).
4. A process according to Claims 1-3, wherein the Hepatitis B surface antigen is Engerix™-B vaccine.
- 25 5. A human monoclonal antibody obtained by the process of Claim 1 and fragments thereof substantially maintaining the antigen binding characteristics of said antibodies.
6. A hybridoma cell line producing a human monoclonal antibody in accordance with Claim 5.
- 30 7. A human monoclonal antibody being selected from the group consisting of:
 - (a) the monoclonal antibody 18.5.1013 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052170;
 - 35 (b) an antibody capable of binding to the antigen which is bound by said 18.5.1013 antibody; and

- (c) fragments of the antibodies of (a) or (b) which substantially retain the antigen binding characteristics of the whole antibodies.
8. A human monoclonal antibody being selected from the group consisting of:
- 5 (a) the monoclonal antibody 19.79.5 which is secreted by the hybridoma cell line deposited in the European Collection of Cell Cultures (ECACC) under Accession No. 96052168;
- (b) an antibody capable of binding to the antigen which is bound by said 19.79.5 antibody; and
- 10 (c) fragments of the antibodies of (a) or (b) which substantially retain the antigen binding characteristics of the whole antibodies.
9. The hybridoma cell line deposited at the ECACC on May 22, 1996 under Accession No. 96052170.
10. The hybridoma cell line deposited at the ECACC on May 22, 1996
- 15 under Accession No. 96052168.
11. An antigen bound by an antibody according to Claim 7 or 8.
12. A pharmaceutical composition for the treatment of HBV infections comprising as an active ingredient an antibody in accordance with Claims 5, 7 or 8 together with a pharmaceutically acceptable carrier.
- 20 13. A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of antibodies according to Claims 5, 7 and 8.
14. A method for the prevention of HBV infections comprising administering to an individual antibodies in accordance with Claims 5, 7 or 8 to prevent further
- 25 infection of the treated individual with HBV.
15. A method for the prevention of HBV infections comprising immunizing an individual with an antigen according to Claim 11.
16. A method for the diagnosis of HBV infections in a body fluid sample comprising:
- 30 (a) contacting said sample with an antibody of any of Claims 5, 7 or 8 under conditions enabling the formation of antibody-antigen complexes;
- (b) determining the level of antibody-antigen complexes formed; a level significantly higher than that formed in a control sample indicating an
- 35 HBV infection in the tested body fluid sample.
17. A method for detecting anti HBV antibodies in a body fluid sample

comprising:

- (a) contacting the sample with the antigen of Claim 11 under conditions enabling the formation of antibody-antigen complexes;
 - (b) determining the level of antibody-antigen complexes formed; and
 - 5 (c) indicating the presence of HBV antigens in the tested body fluid sample.
18. A kit for use in the method of Claim 16.
19. A kit for use in the method of Claim 17.
20. Use of an antibody in accordance with any one of claims 5, 7, or 8 in
- 10 combination with an anti viral agent for the treatment of HBV infection.
21. Use of an antibody in accordance with claim 20 wherein said viral agent is selected from the group consisting of: interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.
- 15 22. A pharmaceutical composition for the treatment of HBV infections comprising as an active ingredient at least one antibody in accordance with claims 5, 7, or 8 adopted for use in combination with at least one other active ingredient being an anti viral agent.
23. A pharmaceutical composition according to claim 22 wherein the anti
- 20 viral agent is selected from the group consisting of: interferons, anti HB monoclonal antibodies, anti HB polyclonal antibodies, nucleoside analogues and inhibitors of DNA polymerase.
24. A method for the treatment of HBV infections comprising administering to an individual in need a therapeutically effective amount of a pharmaceutical
- 25 composition according to claims 22 or 23.

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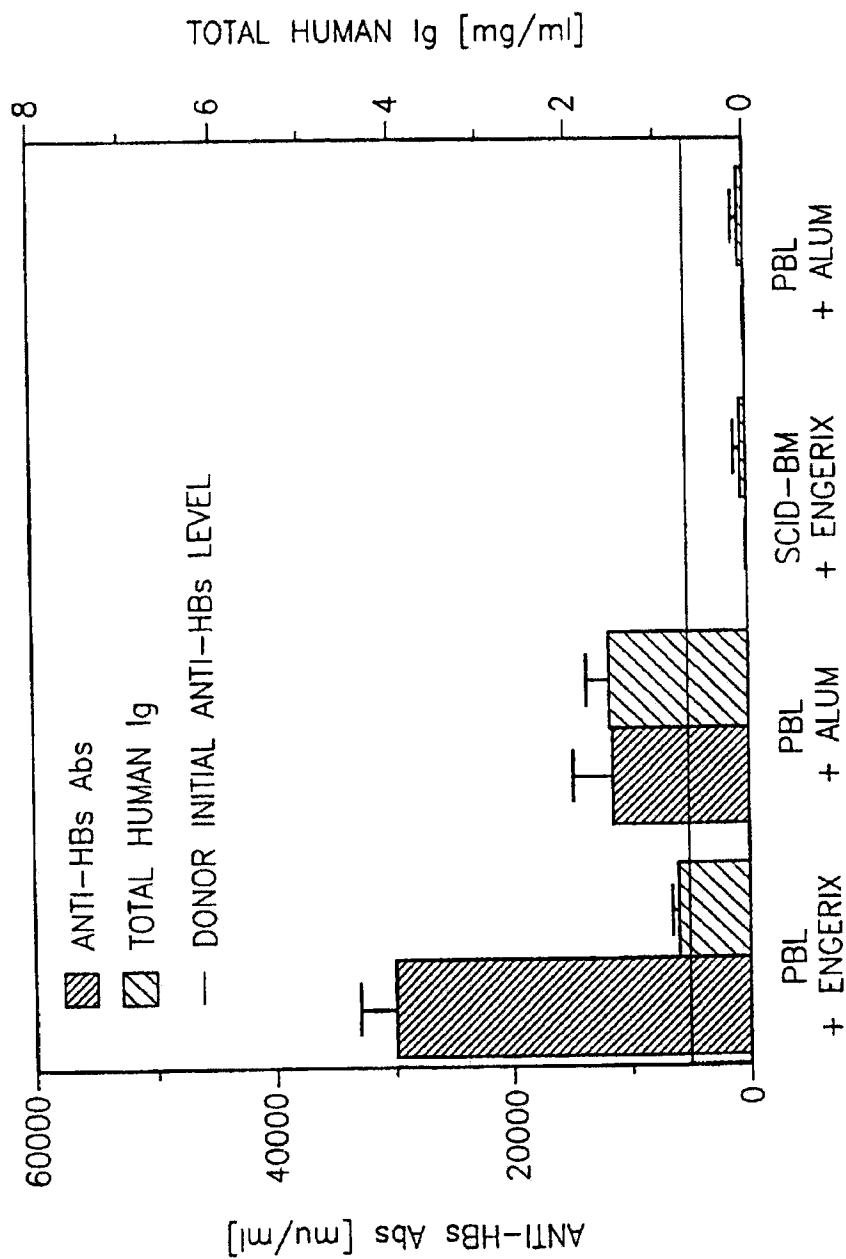


FIG.1

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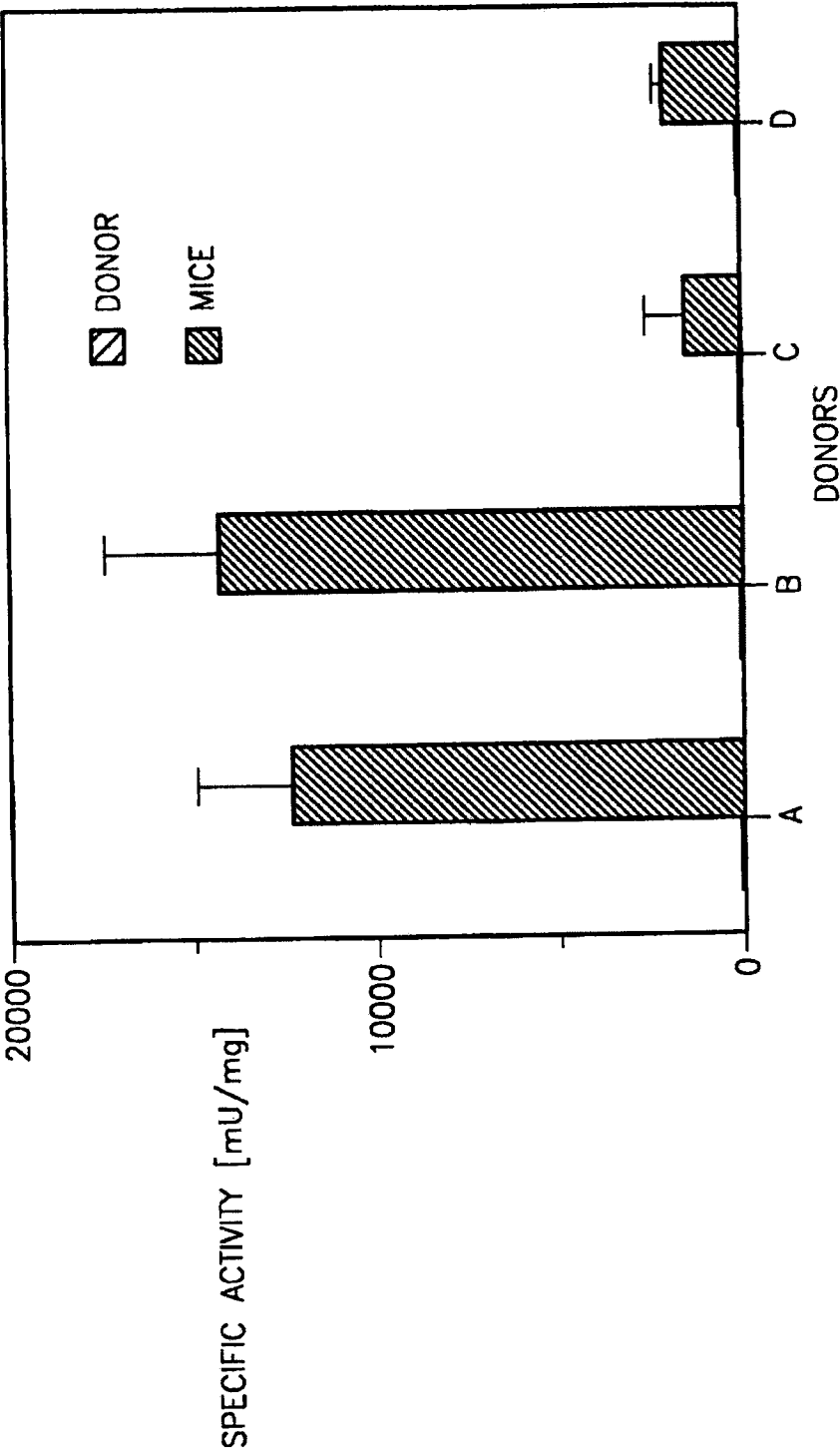


FIG. 2

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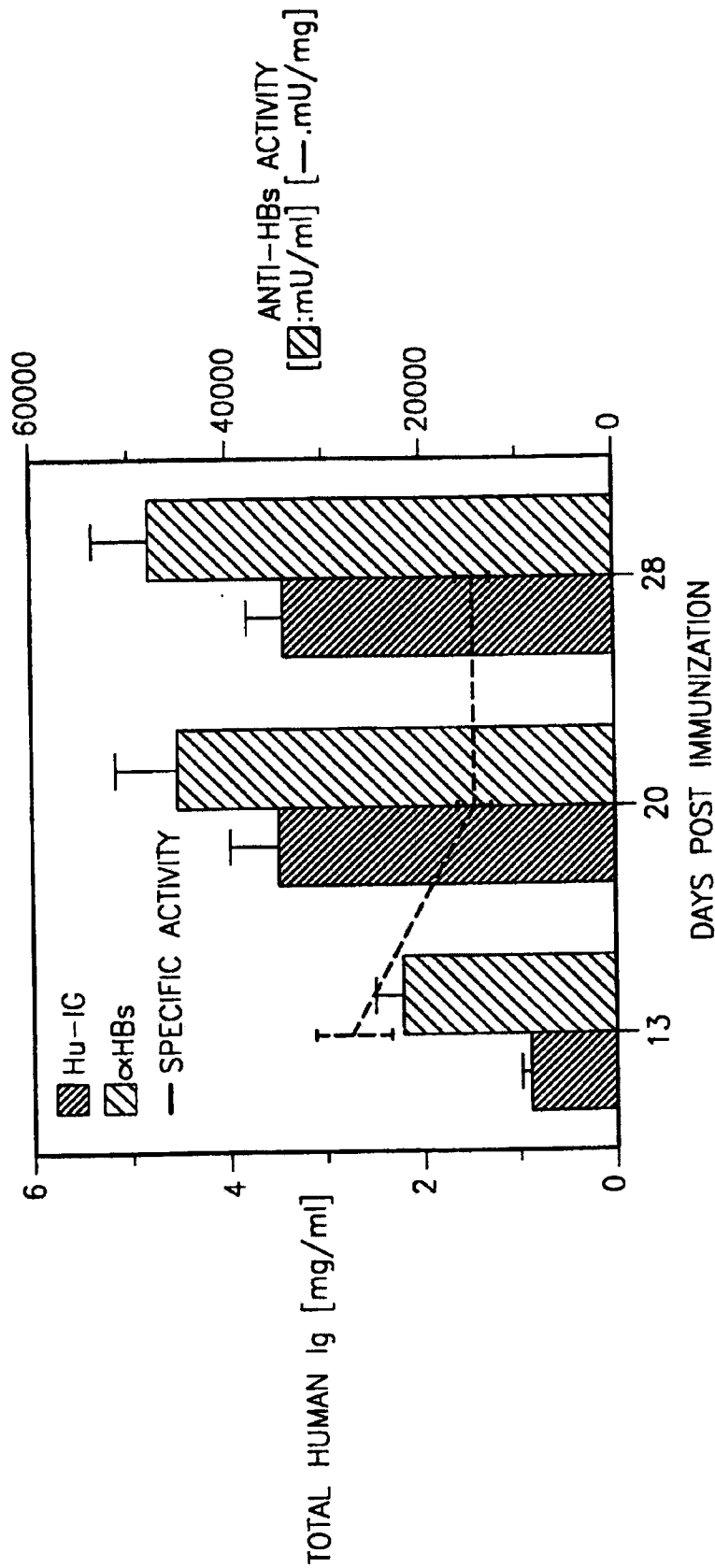


FIG.3

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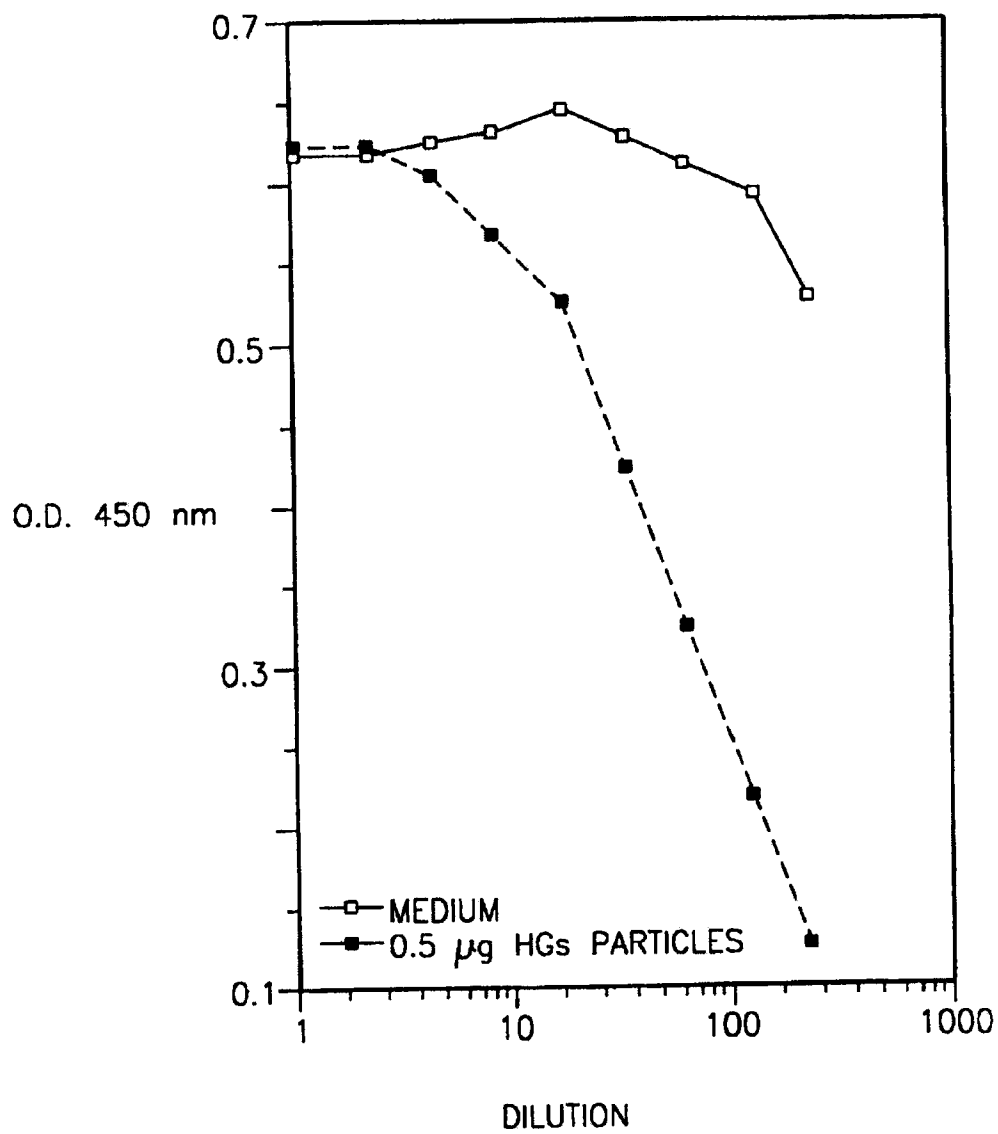


FIG.4

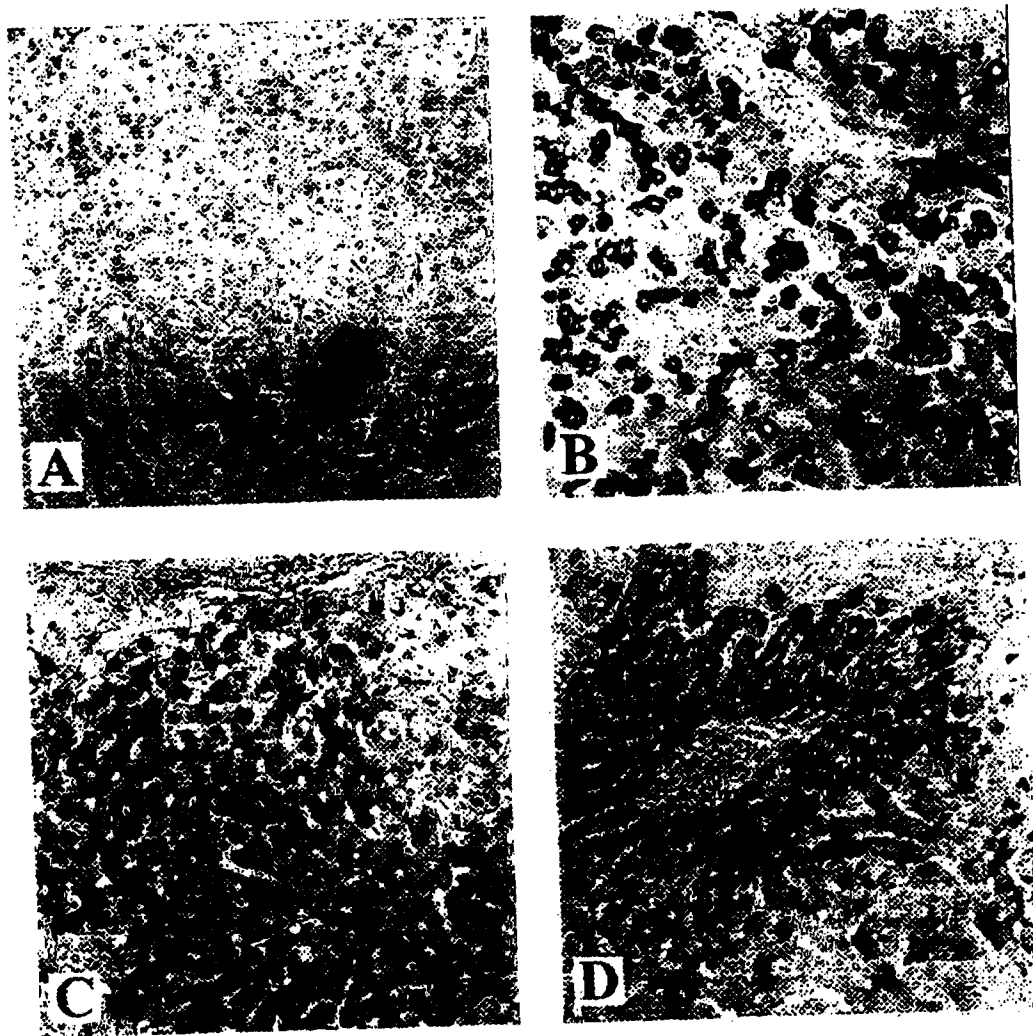


FIG.5

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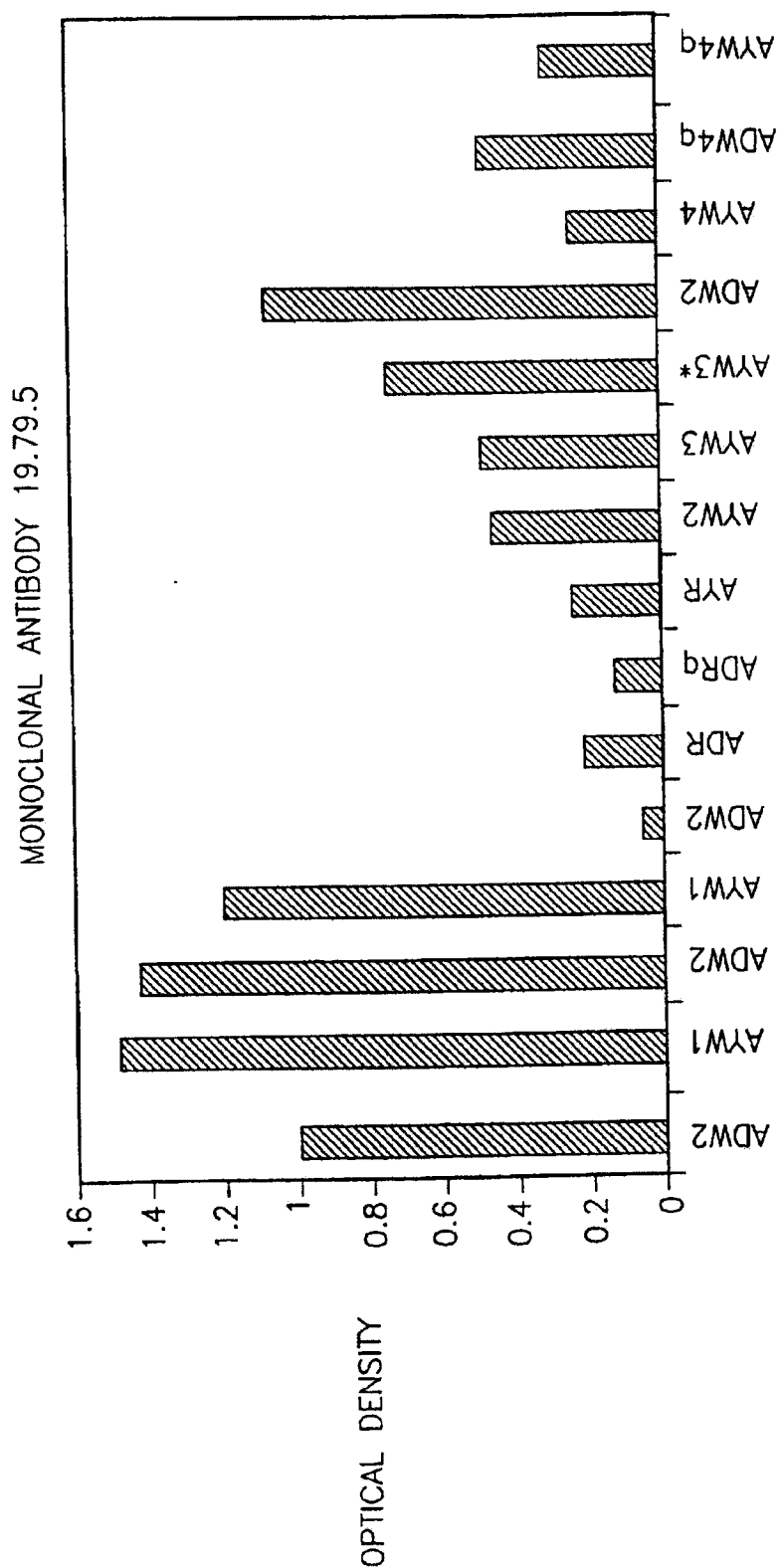


FIG.6

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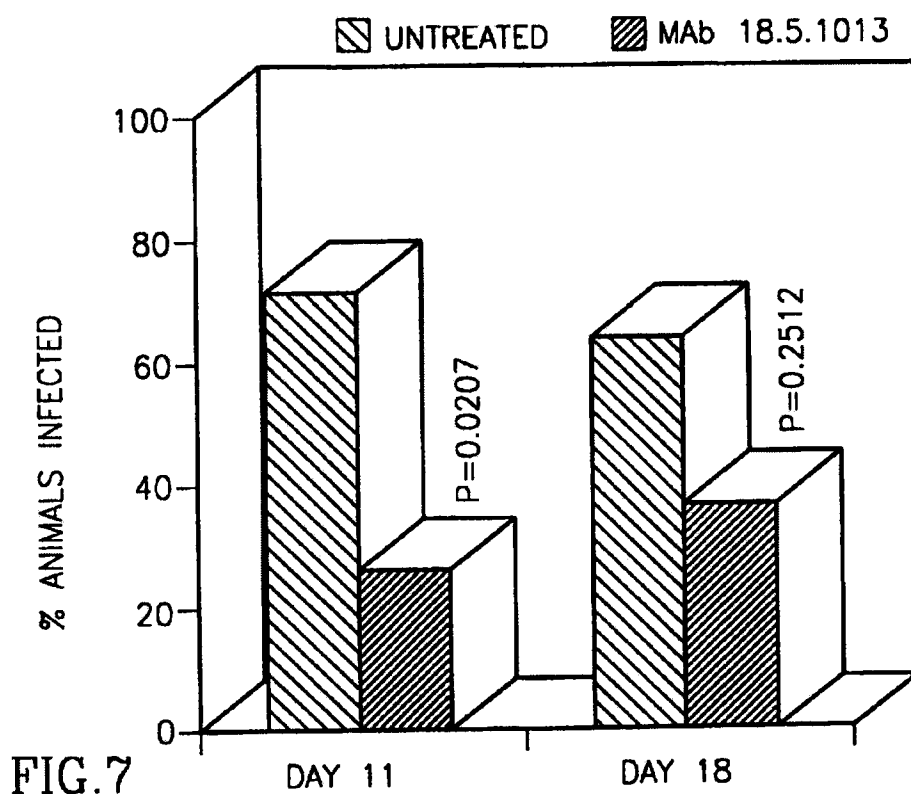


FIG.7

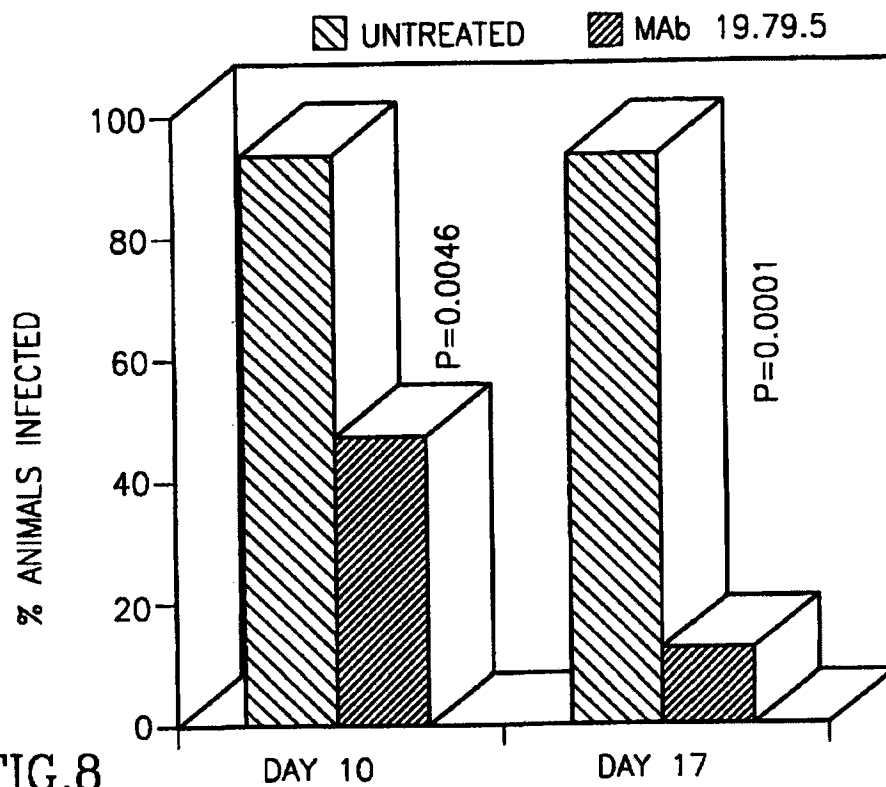


FIG.8

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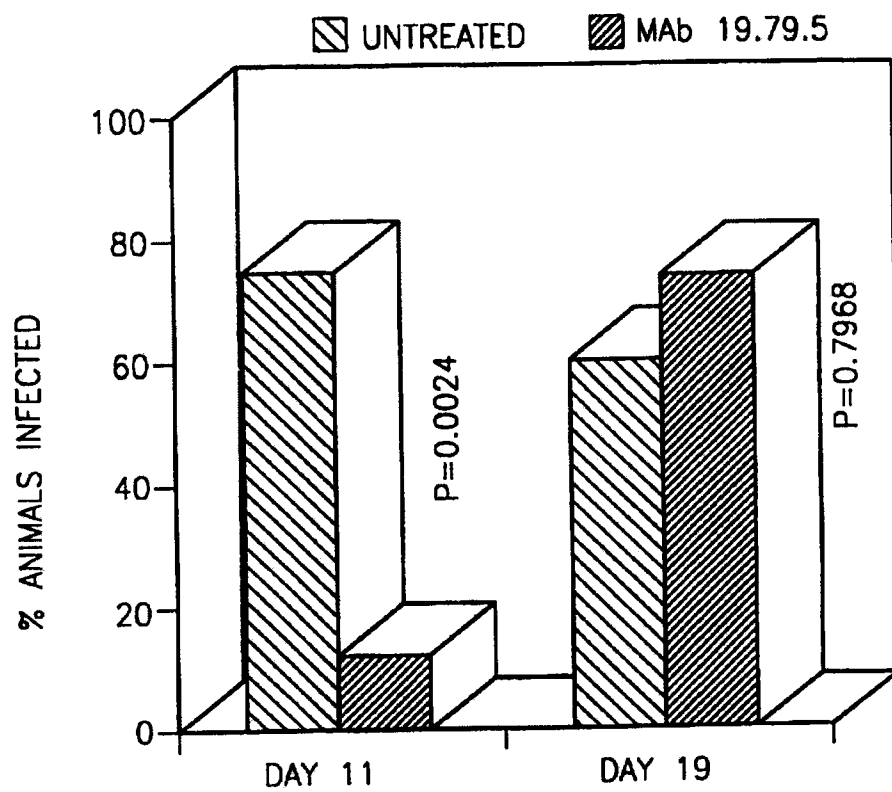


FIG.9

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Arg AGG	Ile ATT	Ser TCC	Tyr TAT	Val GTG	Leu CTG	Thr ACT	Gln CAG	Pro CCA	Pro CCC	Ser TCG	Val GTG	Ser TCA	Val CTG	Ala GCC	Pro CCA	Gly GGA	Lys AAG	Thr ACG	Ala GCC
				9			18			27			36			45			54
Arg AGG	Ile ATT	Ser TCC	Cys TGT	Gly GGG	Gly GGA	Asn AAC	Asn AAC	Ile ATT	Gly GGA	Thr ACT	Lys AAA	Asn AAT	Val GTG	His CAC	Trp TGG	Tyr TAC	Gln CAG	Gln CAG	Lys AAG
		63		72				81			90			99			108		
Pro CCA	Gly GGC	Gln CAG	Ala GCC	Pro CCT	Val GTG	Leu CTG	Val GTC	Val GTC	Tyr TAT	Ala GCT	Asp GAT	Ser AGC	Asp GAC	Arg CGG	Pro CCC	Ser TCA	Gly GGG	Ile ATC	Pro CCT
		123		132				141			150			159			168		
Glu GAG	Arg CGA	Phe TTC	Ser TCT	Gly GGC	Ser TCC	Asn AAC	Ser TCT	Gly GGG	Asn AAC	Thr ACG	Ala GCC	Thr ACC	Leu CTG	Thr ACC	Ile ATC	Ser AGC	Arg AGG	Val GTC	Glu GAA
		183		192				201			210			219			228		
Val GTC	Gly GGG	Asp GAT	Glu GAG	Ala GCC	Asp GAC	Tyr TAT	Tyr TAC	Cys TGT	Gln CAG	Val GTG	Trp TGG	Asp GAT	Ser AGT	Val GTT	Ser AGT	Tyr TAT	His CAT	Val GTG	Val GTA
		243		252				261			270			279			288		
Phe TTT	Gly GGC	Gly GGA	Gly GGG	Thr ACC	Thr ACG	Leu CTG	Thr ACC	Val GTC	Leu CTA	Gly GGT									
		303		312				321											

FIG.10

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Arg	Leu	AGA	CTC	63	Ser	TCC	63	Arg	AGA	CTC	54	Leu	CTG	54
Gln	Val	GTG	9	Gln	CAG	9	Val	GTG	36	Val	GTG	Gly	GGG	45
Leu	Val	GTG	18	Glu	GAG	18	Ser	TCT	27	Gly	GGA	Gly	GGG	45
Pro	Val	GTG	72	Ser	TCT	72	Phe	TTC	81	Val	GTG	Gly	GGG	45
Leu	Val	GTG	132	Glu	GAG	132	Trp	TGG	141	Val	GTG	Gly	GGG	45
Thr	Pro	CCA	123	Ala	GCA	72	Gly	GGA	90	Arg	AGG	Arg	CGC	CGC
Gln	Thr	ACT	123	Lys	AAG	132	Gly	GGA	90	Val	GTG	Trp	TGG	108
Phe	Tyr	TAT	183	Asp	GAC	192	Lys	AAG	201	Arg	AGG	Val	GTG	108
Leu	Tyr	TAT	243	Gln	CAA	252	Met	ATG	261	Ala	GCA	Ala	GCA	261
Glu	Arg	AGG	303	Ile	ATT	312	Pro	CCT	321	Phe	TTC	Thr	CTG	348
Val	Ser	TCC	363	GTC	TCG	363	Val	GTG	363	Val	GTG	Val	GTG	363

FIG.11

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 97/00184

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K16/08 A61K39/29 A61K39/42 C12N5/28 G01N33/569
G01N33/577 //A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A01K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H. MARCUS ET AL.: "Human mouse radiation chimera are capable of mounting a human primary humoral response." BLOOD, vol. 86, no. 1, 1 July 1995, NEW YORK, NY, USA, pages 398-406, XP002044293 cited in the application see the whole document	1-5,7,8, 11-24
Y	---	6,9,10
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "Z" document member of the same patent family

Date of the actual completion of the international search

22 October 1997

Date of mailing of the international search report

10.11.97

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Fax: (+31-70) 340-3016

Authorized officer

Nooij, F

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/IL 97/00184

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	G. KÖHLER AND C. MILSTEIN: "Continuous cultures of fused cells secreting antibody of predefined specificity." NATURE, vol. 256, 7 August 1975, LONDON, GB, pages 495-497, XP002044294 cited in the application see the whole document ---	6,9,10
Y	I. LUBIN ET AL.: "Engraftment of human peripheral blood lymphocytes in normal strains of mice." BLOOD, vol. 83, no. 8, 15 April 1994, NEW YORK, NY, USA, pages 2368-2381, XP002044295 cited in the application see abstract see page 2376, right-hand column, line 53 - line 55 see page 2376, right-hand column, line 58 - page 2377, left-hand column, line 3 see page 2379, right-hand column, line 31 - line 52 ---	1-24
Y	WO 94 11495 A (SANDOZ LTD.) 26 May 1994 cited in the application see the whole document ---	1-24
A	EP 0 438 053 A (YEDA RESEARCH AND DEVELOPMENT CO., LTD.) 24 July 1991 see the whole document ---	1-3
A	L. KULOVA ET AL.: "Natural antibodies do not inhibit xenogeneic transplantation of human PBL in lethally irradiated mice." XENOTRANSPLANTATION, vol. 2, no. 1, 1995, pages 8-18, XP002044296 see the whole document ---	1-3
A	WO 94 26784 A (STICHTING CENTRAAL LABORATORIUM VAN DE BLOEDTRANSFUSIEDIENST) 24 November 1994 cited in the application see the whole document ---	1-24
A	EP 0 179 483 A (CHEMO SERO THERAPEUTIC RESEARCH INSTITUTE) 30 April 1986 see the whole document ---	1-24
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No
PCT/IL 97/00184

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	P. EHRLICH ET AL.: "Characterization of human monoclonal antibodies directed against hepatitis B surface antigen." HUMAN ANTIBODIES AND HYBRIDOMAS, vol. 3, January 1992, pages 2-7, XP000573890 see abstract	1-24
P,0, X	--- R. EREN ET AL.: "Production of specific human monoclonal antibodies to hepatitis B virus by human lymphocytes engrafted in normal strains of mice." JOURNAL OF HEPATOLOGY, vol. 25, no. suppl. 1, 25 - 29 August 1996, page 80 XP002044297 see the whole document -----	1-24

INTERNATIONAL SEARCH REPORT

International application No.
T/IL 97/00184

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 13-15, 20, 21 and 24 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/IL 97/00184

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 179483 A	30-04-86	JP 61104796 A CA 1339202 A DE 3584872 A US 4883752 A	23-05-86 05-08-97 23-01-92 28-11-89